Rheological and Functional Properties of Catfish Skin Protein Hydrolysates

HUAIXIA YIN, JIANING PU, YUTING WAN, BOB XIANG, PETER J. BECHTEL, AND SUBRAMANIAM SATHIVEL

ABSTRACT: Catfish skin is an abundant and underutilized resource that can be used as a unique protein source to make fish skin protein hydrolysates. The objectives of this study were to isolate soluble and insoluble proteins from hydrolyzed catfish skin, study the rheological and functional properties of the protein hydrolysates, and evaluate the properties of emulsions made from the protein powders. Freeze-dried catfish skin soluble (CSSH) and insoluble hydrolysate (CSISH) powders were analyzed for proximate analysis, emulsion stability, fat absorption, amino acids, color, and rheological properties. CSSH had significantly ($P < 0.05$) higher protein, ash, and moisture content but lower fat content than that of CSISH. The yield of CSSH (21.5% ± 2.2%) was higher than that of CSISH (3% ± 0.3%). CSISH had higher emulsion stability than CSSH. CSSH was light yellow in color and CSISH was darker. The mean flow index values for emulsion containing CSSH (ECSSH) and CSISH (ECSISH) were both less than 1, indicating that they were both pseudoplastic fluid. The G' and G'' values for the ECSISH were higher than that of ECSSH, indicating that the viscoelastic characteristic of the emulsion containing CSISH was greater than that of the emulsion containing CSSH. The study demonstrated the CSSH and CSISH had good functional and rheological properties. They have potential uses as functional food ingredients.

Keywords: catfish, emulsion, functional properties, protein hydrolysates, rheological properties

Introduction

Channel catfish is the 4th-most popular fish product consumed in the United States. The 4 major commercial catfish producing states in the United States are Alabama, Arkansas, Louisiana, and Mississippi. In 2005, these states produced over 272000 metric tons catfish with a stable monthly production of about 22700 tons (NASS 2006). The by-products of catfish processing consist of heads, frames, skin, and viscera, which often end up in landfills or rendering plants.

The yield of catfish when processed as whole fillets is around 45%, generating about 55% byproducts, which includes 6% skin. In 2005, the estimated amount of catfish skin produced from fish processors in Alabama, Arkansas, Louisiana, and Mississippi was 16320 metric tons. Catfish skin protein hydrolysates can be prepared using proteolytic enzymes that have a variety of chemical and functional properties. Catfish skin hydrolysates could be converted into high value food ingredients and added to meat products to improve functional properties, and as unique protein extenders in food products.

Rheological properties of emulsion provide valuable information that can be used in quality control of storage stability, sensory assessments of consistency, and design of product texture and unit operations and are one of the most important qualities of mayonnaise and salad dressing-type products (Davis 1973). Most emulsions exhibit both solid (elasticity) and fluid (viscosity) behavior when they are subjected to an instantaneous shear stress, which is maintained constant for a sufficient time (Sherman 1970). Several rheological equations, such as the power law, the Casson model, and the Herschel–Bulkley model, have been used to describe the stress response to deformation in emulsion (Paredes and others 1989). Flow parameters such as consistency index, flow behavior index, and yield stress are different from one emulsion to another due to the type of emulsifier and other ingredients used, and selected rheological measuring ranges.

Fish protein powders have a range of functional properties, and can potentially be used in foods as emulsifiers (Sathivel and others 2004). Amino acids and peptides are increasingly being used in energy drinks and other food applications (O'Donnell and Dornblaser 2002). Egg yolk is commonly used as an emulsifier in many foods (Paredes and others 1989); however, a single egg yolk may contain up to 210 mg of cholesterol. The recommendation for cholesterol intake is no more than 300 mg/d (Wardlaw and Insel 1995). Salad dressing and mayonnaise are oil-in-water emulsions containing basic ingredients (vegetable oil, egg yolk, vinegar, lemon juice) and additives (egg white, salt, spices, stabilizers, thickeners, and so on). Arrowtooth flounder protein powder provide desirable emulsifying properties in the mayonnaise system that exhibits pseudoplastic and viscoelastic characteristics (Sathivel and others 2005b). It may be possible to substitute egg yolk with fish protein hydrolysates in an oil-in-water emulsion system.

Dried and processed fish proteins are not widely used because of the loss of protein functionality as a result of processing and the sensory properties of oxidized fish lipid. Shahidi (1994) has reported that enzymatic hydrolysis can be used to improve the quality and functional characteristics of fish processing byproducts. A number of fish processing byproducts hydrolysates have been reported in the scientific literature including barramundi (*Lates calcarifer*) larvae (Nankervis and Southgate 2009), persian sturgeon (*Acipenser persicus*) viscera (Ovissipour and others 2009), hoki fish (*Johnius belengerii*) skin (Mendis and others 2005), and wastes

MS 20090559 Submitted 6/17/2009, Accepted 8/8/2009. *Authors Yin, Pu, Wan, Xiang, and Sathivel are with Dept. of Food Science, Louisiana State Univ. Agricultural Center, Baton Rouge, LA 70803-4300, U.S.A. Author Bechtel is with USDA/ARS Subarctic Research Unit, 245 O'Neill Building, Univ. of Alaska, Fairbanks, AK 99775, U.S.A. Direct inquiries to author Sathivel (E-mail: ssathivel@agcenter.lsu.edu).*

Approved for publication by the director of the Louisiana Agricultural Experimental Station as manuscript nr 2009-232-3628.

doi: 10.1111/j.1750-3841.2009.01385.x

from cod (*Gadus morhua*), shrimp (*Pandalus borealis*), and sardine (*Sardina pilchardus*) processing industries (Bordenave and others 2002).

Enzymatic hydrolysis can be used to improve or modify the physicochemical, functional, and/or sensory properties of native proteins without losing nutritional value, especially partial hydrolysis, is often employed to improve the functionalities of food proteins (Althouse and others 1995; Kuipers and others 2005). The functionalities of fish proteins hydrolysates are depended on the protein sources, proteases used (Sugiyama and others 1991), degree of hydrolysis, buffer pH (Doucet and others 2003), and reaction temperature and time (Feng and Xiong 2003), while type of proteolytic enzyme used and hydrolysis reaction conditions influence the sensory characteristics of hydrolysates such as bitterness (Lin and others 1997). A number of proteolytic enzymes, including commercially available exo- and endopeptidases, can be used to make hydrolysates. Hydrolysates produced using an endopeptidase (alcalase) from Nile perch (*Lates niloticus*), Grass Carp (*Ctenopharyngodon idella*), and Nile tilapia (*Oreochromis niloticus*) skin exhibited good functional properties (Wasswa and others 2008).

Fish protein hydrolysate has been reported to have antioxidant properties (Sathivel and others 2003; Mendis and others 2005). Shahidi and Amarowicz (1996) have reported the antioxidant activity of protein hydrolysates from 2 aquatic species, namely capelin and harp seal. Sathivel and others (2008) have reported that coating solution prepared from pollock skin hydrolysate acts as antioxidation agent and delayed the lipid oxidation of the pink salmon fillets during 4 mo frozen storage. Peptides generated during hydrolysis coupled with free amino acids could be responsible for antioxidant activity (Chen and Decker 1994). Yang and others (2007) used alkaline and acid methods to extract the catfish skin gelatin. Catfish fillets hydrolysates produced using the enzyme Protamex were investigated for their antioxidative properties (Theodore and others 2008). However, there is not much information available on catfish skin protein hydrolysates and their rheological and functional properties. The objectives of study were to isolate soluble and insoluble protein hydrolysates from catfish skin and study the rheological and functional properties of the hydrolysates.

Materials and Methods

Preparation of catfish fish protein hydrolysates

Skin from Channel catfish (*Ictalurus punctatus*) was obtained from a local seafood store in Baton Rouge, Louisiana, and stored at −40 °C until further processed. The skin was thawed overnight at 4 °C and ground with a Hobart grinder (K5SS, Hobart Corp., Troy, Ohio, U.S.A.) through a plate with 5 mm diameter holes. Hydrolysis conditions were similar to those of Hoyle and Merritt (1994) with minor modifications. A 500 g portion of ground catfish skin was mixed with an equal volume of distilled water at 25 °C and stirred for 2 min. The mixture was then adjusted to pH 8 with sodium hydroxide and the temperature was brought to 50 °C in a water bath for optimal alcalase activity (Novo Nordisk 1995). The alcalase enzyme (> 0.24 U/g) purchased from the Novo Nordisk (Franklinton, N.C., U.S.A.) was added to the minced skin at 0.5% w of protein weight. The mixture was stirred for 45 min at 50 °C and the enzyme was inactivated by increasing the temperature to 85 °C for 15 min. The soluble fraction of hydrolysate was recovered by centrifugation at 2560 × *g* for 15 min and the soluble aqueous fraction decanted, freeze-dried, sealed in vacuum bags, and stored at 4 °C until used. The insoluble fraction (precipitate) was also freeze dried, sealed in vacuum bags, and stored at 4 °C until used. The experiment was replicated 3 times.

Proximate composition

The catfish skin soluble hydrolysates (CSSH) and the catfish skin insoluble hydrolysates (CSISH) samples were analyzed in triplicate for moisture and ash using the AOAC standard methods 930.15 and 942.05, respectively (AOAC 1995). Fat content was determined using dichloromethyl ether in automated ASE-200 fat extractor (Dionex Corp., Sunnyvale, Calif., U.S.A.). The nitrogen content was determined in triplicate using the Leco FP-2000 Nitrogen Analyzer (LECO Corp., St. Joseph, Mich., U.S.A.). The protein, fat, lipid, and ash contents of CSSH and CSISH were reported as wet basis. The yield was calculated by determining the dried catfish skin protein hydrolysate powder weight as a percentage of the total raw material wet weight (Hoyle and Merritt 1994).

Amino acid and mineral analysis

Amino acid profiles were determined by the AAA Service Lab. Inc. (Boring, Oreg, U.S.A.). Samples were hydrolyzed with 6 N HCl and 2% phenol at 110 °C for 22 h. Amino acids were quantified using a Beckman 6300 analyzer with post-column ninhydrin derivatization. Tryptophan and cysteine content were not determined.

CSSH and CSISH samples for mineral analysis were ashed overnight at 550 °C. Ashing residues were digested overnight in an aqueous solution containing 10% (v/v) hydrochloric acid and 10% (v/v) nitric acid. Digested solutions were diluted as needed and analyzed for B, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Sr, S, and Zn by inductively coupled plasma optical emission spectroscopy on a Perkin Elmer Optima 3000 Radial ICP−OES (PerkinElmer Life and Analytical Sciences Inc., Boston, Mass., U.S.A.).

Color, SDS-PAGE electrophoresis of CSSH and CSISH

Color of the powders were determined using a LabScan® XE spectrophotometer (Hunter Associates Lab. Inc., Resbon, Va., U.S.A.) and reported as L^*, a^*, and b^* (L^* value is degree of lightness to darkness, a^* value is degree of redness to greenness, and b^* value is degree of yellowness to blueness). Chroma and hue angle were calculated. The SDS tricine/polyacrylaminde gel electrophoresis system was used with a Photodyne Foto/Force 300 power supply (Hartland, Wis., U.S.A.) and a single sided vertical gel electrophoresis system (Owl Separation Systems, Portsmouth, N.H., U.S.A.) under reducing conditions according to Schagger and Von Jagow (1987). A 10 mg/mL sample was prepared for electrophoresis in an equal volume of tris buffer containing 20% sodium dodecyl sulfate, 50% glycerol, and bromophenol blue. Then 2-mercatoethanol was added (5% of final volume), the sample mixed vigorously and then heated to 95 °C for 10 min. Novex Precast 10% to 20% Tricine gels (Invitrogen Life Technologies, Carlsbad, Calif., U.S.A.) were used and ColorBurst molecular mass standards were purchased from Sigma-Aldrich (Number C 4105, St. Louis, Mo., U.S.A.). The load per lane on the gel was 25 ug.

Protein bands were visualized by staining the gels with Coomassie blue.

Functional properties of catfish skin protein hydrolysates

The nitrogen solubility was determined following the procedure of Morr and others (1985). A 500 mg of each CSSH or CSISH sample was dispersed in 50 mL of 0.1 M NaCl at pH 7 and the solution stirred for 1 h at 25 °C and centrifuged at 2560 × *g* for 30 min. The supernatant was analyzed for nitrogen using the Leco FP-2000 Nitrogen Analyzer. The solubility of CSSH or CSISH, defined as the

amount of soluble nitrogen from the total nitrogen, was calculated as:

$$\text{Nitrogen solubility (\%)} = \frac{\text{Supernatant nitrogen concentration}}{\text{Sample nitrogen concentration}} \times 100$$

Emulsifying stability was measured by an oil titration method similar to that of Yatsumatsu and others (1972). A 500 mg of CSSH or CSISH samples was dissolved in 50 mL of 0.1 M NaCl solution in a tared 250 mL beaker, and then 50 mL of soybean oil were added. The homogenizer (model 6-105-AF, Virtis Co, Gardner, N.Y., U.S.A.) equipped with a motorized stirrer driven by the rheostat was immersed in the mixture and operated for 2 min at 100% output at 120 V to make an emulsion. From the emulsion, three 25-mL aliquots were immediately taken and transferred into three 25-mL graduated cylinders. The emulsions were allowed to stand for 15 min at 25 °C, and then the aqueous volume was read. ES (%) was calculated as ([total volume – aqueous volume]/total volume) × 100.

The fat absorption (FA) of the CSSH or CSISH samples was measured according to the method of Shahidi and others (1995). A 500 mg sample of CSSH or CSISH was put into a 50 mL centrifuge tube and 10 mL soybean oil was added. The sample was thoroughly mixed with a small steel spatula, kept for 30 min at 25 °C with mixing every 10 min, and then centrifuged for 25 min at 2560 × *g*. Free oil was then decanted and the fat absorption of the sample determined from the weight difference between the catfish protein hydrolysate after the adsorption and before the adsorption. The fat absorption was expressed in terms of milliliters of fat absorbed by 1 g of protein.

Preparation of emulsions containing CSSH or CSISH

Emulsions containing catfish skin protein hydrolysates were prepared with CSSH or CSISH (5%, w/w), soybean oil (60.35%, w/w), water (30%, w/w), salt (3.6%, w/w), lemon juice (1%, w/w), and xanthan gum (0.05%, w/w). The emulsions were made by adding 30 g of water and 3.6 g of salt to a 400-mL beaker, which was mixed for 2 min using an ultrasonic processor. A Vibra cell Ultrasonic Processor, converter model CV33, equipped with a 13-mm probe (Sonics, Newtown, Conn., U.S.A.) was used at 80% amplitude with 2 × 1 s pulses (with 1 s delay between pulses) and samples were held in an ice bath during the procedure. Then xanthan gum (0.05 g) was added to the salt solution and sonicated for 2 min at 4° C. This was followed by the addition of 5 g of CSSH or CSISH and 3 min of additional sonication, 60.35 g soybean oil was added to the beaker and emulsion formed by sonication for 15 min, followed by the addition of the lemon juice and stirred an additional 2 min of sonication.

Oil recovery, color, and rheological properties of emulsion containing CSSH and CSISH

Oil recovery of the emulsion was evaluated according to the method of Min and others (2003) with minor modifications. Emulsion samples (5 g) made by CSSH and CSISH were placed into a 10 mL centrifugal tube and stored at −20 °C for 2 d and then allowed to thaw at room temperature for 1 h. The thawed samples were centrifuged at 15000 × *g* for 40 min at −2 °C and the amount of oil separated measured. Oil recovery percent was calculated as (weight of oil recovered/5 g of emulsion sample) × 100.

Color analyses of the emulsion samples were performed using a LabScan® XE colorimeter (Hunter Associates Lab. Inc.) and reported as L^*, a^*, and b^*. Rheological properties of the emulsion samples were measured using an AR 2000 Rheometer (TA Instruments, New Castle, Del., U.S.A.) fitted with a plate geometry (acrylic plates with a 40-mm diameter having a 200 μm gap between the 2 plates). Each sample was placed in the temperature-controlled parallel plate and allowed to equilibrate to 25 ± 0.1 °C. Shear stress was measured at varying shear rates from 0 to 200 s^{-1}. The mean values of triplicate samples on n (flow behavior index), K (consistency index), and viscosity (shear rate of 200 s^{-1}) were reported.

The power law (Eq. 1) and the Casson equation (Eq. 2) were used to analyze the flow properties of the emulsion samples.

$$\sigma = K\gamma^n \tag{1}$$

where σ = shear stress (Pa), γ = shear rate (s^{-1}), K = consistency index (Pa.s^n), and n = flow behavior index.

$$\sigma^{0.5} = \sigma_o^{0.5} + K\gamma^{0.5} \tag{2}$$

where K = consistency coefficient and σ_o = yield stress (Pa).

Viscoelastic properties of the emulsion were measured using an AR 2000 Rheometer (TA Instruments) fitted with a plate geometry (acrylic plates with a 40-mm diameter having a 200 μm gap between the 2 plates). Each sample was placed on the parallel plate and the frequency sweep test was conducted at a constant temperature of 25 °C to determine the viscoelastic properties of emulsion containing CSSH and CSISH. Viscoelastic properties of emulsions were characterized using the Eq. (3) and (4).

$$G' = \left[\frac{\sigma_o}{\gamma_o}\right]\cos\delta \tag{3}$$

$$G'' = \left[\frac{\sigma_o}{\gamma_o}\right]\sin\delta \tag{4}$$

where G' = storage modulus (Pa) and G'' = loss modulus (Pa).

Statistical analysis

Mean values and standard deviations (SD) from the 3 separate experiments or replicate analysis were reported. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA), followed by the post hoc Tukey's studentized range test (SAS 2002).

Results and Discussion

Proximate composition

The proximate composition of freeze dried CSSH and CSISH powders is given in Table 1. The CSSH had significantly higher protein content (88.34%) than that of CSISH (39.73%). The fat content for CSSH (1.83%) was significantly ($P < 0.05$) lower than CSISH (52.28%). The moisture and ash content of CSSH (6.75% and 3.07%) were significantly higher than those of CSISH (6.10% and 1.89%). The yield of CSSH (21.51%) was much higher ($P < 0.05$)

Table 1 — Proximate composition and yield of CSSH and CSISH.

Composition (%)	CSSH	CSISH
Protein	88.34 ± 0.21[a]	39.73 ± 1.38[b]
Fat	1.83 ± 0.35[b]	52.28 ± 1.60[a]
Moisture	6.75 ± 0.11[a]	6.10 ± 0.17[b]
Ash	3.07 ± 0.18[a]	1.89 ± 0.20[b]
Yield	21.51 ± 2.2[a]	3.03 ± 0.3[b]

Values are means and SD of triplicate determinations.
[ab]Means with different letters in each row are significantly different ($P < 0.05$).
CSSH = catfish skin soluble hydrolysate; CSISH = catfish skin insoluble hydrolysate.

than CSISH (3.03%), possibly indicating a high degree of hydrolysis. Sathivel and others (2005b) reported that the soluble fraction extracted without enzyme from arrowtooth flounder tissues ranged from 8.6% to 13.1%. A number of factors, such as temperature, pH, hydrolysis time, and type of enzymes, affect the degree of hydrolysis efficiency.

Amino acid analysis and mineral content

Protein quality can be evaluated using biological methods such as the protein efficiency ratio, net protein utilization, or chemical methods. In this study, the essential amino acid content of the powders was compared with the recommendations made by FAO/WHO (1990) for adult humans. Most of the soluble and insoluble catfish skin protein hydrolysates met or exceeded the essential amino acid requirements for adult humans except the methionine (Table 2). Total essential amino acids of the CSSH was lower (23%) than CSISH (26.06%) and both of these values were lower than values for red salmon hydrolysate (36.3%) produced using alcalase (Sathivel and others 2005a) and higher than pollock skin hydrolysates (18.3%) (Sathivel and others 2008). The lower amount of essential amino acids in the skin protein hydrolysates might be due to the greater contents of hydroxyproline, proline, and alanine in the fish skin.

The ash contents of the 2 fractions were 3.07% and 1.9% for CSSH and CSISH, respectively (Table 1). As expected, the calcium content of both fractions was low due to the absence of bone in the catfish skin samples; however, similar levels of P were detected in both fractions (Table 3). Higher amount of Na was found in CSSH due to their solubility in the aqueous phase, which was then concentrated by freeze drying. High levels of Zn (472 ppm) and Fe (366 ppm) were found in CSISH, possibly indicating they were undigested to protein fragments or membrane fragments in this fraction.

Color and sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The CSSH was light yellow (L^* = 77.38, b^* = 6.46) in color (Table 4). CSISH was much darker (L^* = 18.74) than CSSH (L^* = 77.38). The CSSH exhibited significantly higher yellowish color, red color, chroma, and hue angle than those of CSISH. The molecular weights of the CSSH and CSISH, which were hydrolyzed for 45 min, were analyzed by SDS-PAGE electrophoresis. Most of the material that stained with commassie blue molecular weight was below 10 kDa for both CSSH and CSISH samples. There was a lack of higher molecular weight bands consistent with a very high degree of hydrolysis. The CSSH and CSISH samples had less defined bands.

Functional properties

Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Mahmoud and others 1992). Table 4 shows nitrogen solubility values for the CSSH and CSISH. The CSSH had much higher nitrogen solubility (99.8%) than CSISH (2.40%). The nitrogen solubility values for CSSH were higher than values reported for red salmon hydrolysates (17.2% to 54.4%) (Sathivel and others 2005a), herring hydrolysates (84.9%) (Sathivel and others 2003), and hydrolyzed herring muscle protein (89.7% to 93.1%) (Hoyle and Merritt 1994). The high solubility is due in part to cleavage of proteins into smaller peptide units which usually increases the nitrogen solubility (Shahidi 1994). The high nitrogen solubility of CSSH indicates potential applications in formulated food systems by providing attractive appearance and smooth mouthfeel to the product (Petersen 1981).

Emulsion stability for CSSH and CSISH was 79% and 99%, respectively (Table 4). CSISH had significantly higher emulsion stability (99%) than CSSH (79%), which might be due to higher amount of lipid content (52.28%) in the insoluble protein hydrolysate. The emulsion stability of hydrolyzed Atlantic salmon muscle protein

Table 2—Amino acid composition of soluble and insoluble protein hydrolysates from catfish skin.

Amino acid	CSSH (%)	CSISH (%)	EAA (%)[A]
Hydroxyproline	6.28 ± 0.03[a]	3.00 ± 0.12[b]	
Aspartic acid	7.53 ± 0.04[b]	8.87 ± 0.07[a]	
Threonine[B]	3.04 ± 0.01[b]	3.91 ± 0.09[a]	0.9
Serine	3.11 ± 0.03[b]	3.40 ± 0.16[a]	
Glutamic acid	11.68 ± 0.04[b]	11.80 ± 0.15[a]	
Proline	9.49 ± 0.03[a]	5.58 ± 0.39[b]	
Glycine	18.70 ± 0.10[a]	11.94 ± 0.26[b]	
Alanine	8.94 ± 0.05[a]	6.56 ± 0.11[b]	
Valine[B]	3.67 ± 0.03[b]	5.39 ± 0.05[a]	1.3
Methionine[B]	0.44 ± 0.02[b]	1.35 ± 0.02[a]	1.7[C]
Isoleucine[B]	2.61 ± 0.01[b]	4.78 ± 0.03[a]	1.3
Leucine[B]	4.42 ± 0.02[b]	7.38 ± 0.03[a]	1.9
Tyrosine	1.77 ± 0.00[b]	3.74 ± 0.00[a]	
Phenylalanine[B]	2.61 ± 0.41[b]	4.69 ± 0.03[a]	
Histidine[B]	1.59 ± 0.00[b]	2.49 ± 0..03[a]	1.6
Hydroxylysine	0.70 ± 0.00[a]	0.66 ± 0.20[a]	
Lysine[B]	4.62 ± 0.04[b]	6.05 ± 0.11[a]	1.6
Arginine	8.81 ± 0.05[a]	8.43 ± 0.12[b]	

Values are means and SD of triplicate determinations.
[ab]Means with different letters in each row are significantly different ($P < 0.05$).
CSSH = catfish skin soluble hydrolysate; CSISH = catfish skin insoluble hydrolysate.
[A]Suggested profile of essential amino acid requirements for adult humans by FAO/WHO (1990).
[B]Essential amino acids.
[C]Methionine + cysteine.

Table 3—Mineral content of CSSH and CSISH.

	CSSH	CSISH
P (%)	0.23 ± 0.01[a]	0.25 ± 0.01[a]
K (%)	0.51 ± 0.03[a]	0.09 ± 0.01[b]
Ca (%)	0.03 ± 0.01[b]	0.06 ± 0.01[a]
Mg (%)	0.03 ± 0.00[a]	0.01 ± 0.00[b]
Na (ppm)	4488.7 ± 46.5[a]	848.7 ± 44.5[b]
Cu (ppm)	0.44 ± 0.09[b]	0.89 ± 0.31[a]
Zn (ppm)	148.3 ± 5.5[b]	472.3 ± 11.4[a]
Mn (ppm)	<1	<1
Fe (ppm)	6.3 ± 2.3[b]	366.7 ± 5.0[a]
S (ppm)	0.35 ± 0.01[a]	0.2 ± 0.01[b]
Cd (ppm)	<0.01	<0.01
Sr (ppm)	0.40 ± 0.12[b]	0.80 ± 0.08[a]
B (ppm)	423.4 ± 26.8[a]	295.3 ± 43.1[b]

Values are means and SD of triplicate determinations.
[ab]Means with different letters in each row are significantly different ($P < 0.05$).
CSSH = catfish skin soluble hydrolysate; CSISH = catfish skin insoluble hydrolysate.

Table 4—Color and functional properties of the CSSH and CSISH.

	CSSH	CSISH
L^*	77.38 ± 0.19[a]	18.74 ± 0.08[b]
a^*	0.84 ± 0.02[a]	0.33 ± 0.01[b]
b^*	6.46 ± 0.05[a]	1.39 ± 0.08[b]
Chroma	6.48 ± 0.04[a]	1.40 ± 0.08[b]
Hue angle	101.67 ± 0.17[a]	76.25 ± 1.09[b]
Nitrogen solubility (%)	99.80 ± 6.91[a]	2.40 ± 0.76[b]
Emulsion stability (%)	79.00 ± 1.73[b]	99.00 ± 0.25[a]
Fat adsorption capacity (mg of oil per g of protein)	9.70 ± 0.26[a]	3.15 ± 0.19[b]

Values are means and SD of triplicate determinations.
[ab]Means with different letters in each row are significantly different ($P < 0.05$).
CSSH = catfish skin soluble hydrolysate emulsion; CSISH = catfish skin insoluble hydrolysate emulsion.

was reported to be 52% to 61% and that of the hydrolyzed herring byproducts to be 48.6% to 54.2%. Difference in emulsifying properties of hydrolysates may be due to differences in hydrophobicity (Gauthier and others 1993) and peptide lengths (Jost and others 1977). Smaller peptides often have reduced emulsifying properties (Chobert and others 1988). A positive correlation between surface activity and peptide length has been reported (Jost and others 1977). Lee and others (1987) found a peptide should have a minimum length of 20 residues to possess good emulsifying and interfacial properties. Fat adsorption capacity is an important functional characteristic of ingredients used in the meat and confectionary industries. As shown in Table 4, CSSH had a higher fat absorption capacity (9.7 mL/g protein) than CSISH (3.15 mL/g of protein). A previous study reported a fat absorption capacity range of 2.86 to 7.07 mL of oil/g protein for Atlantic salmon hydrolysate, and Sathivel and others (2005b) reported values of 3.3 to 5.2 mL of oil/g of protein for arrowtooth flounder fillets.

Oil recovery and color of emulsions containing CSSH and CSISH

The oil recovery of the emulsion containing CSSH (54.4%) was higher than the ECSISH (43.58%) (Table 5). Oil recovery of the emulsions is in agreement with the emulsion stability of the hydrolysates (Table 4). An emulsion with higher emulsion stability shows less oil recovery. The emulsion containing the catfish skin soluble hydrolysates (ECSSH) was in light yellow color ($L^* = 71.90$, $b^* = 16.96$) compared with the color of the emulsion containing catfish skin insoluble hydrolysates (ECSISH) ($L^* = 35.83$, $b^* = 7.71$). The ECSISH showed higher red color and lower chroma and hue angle than those of ECSSH.

Table 5 — Oil recovery and color of ECSSH and ECSISH.

	ECSSH	ECSISH
Oil recovery (%)	54.4 ± 0.25[a]	43.58 ± 0.38[b]
L^*	71.90 ± 0.01[a]	35.83 ± 0.16[b]
a^*	1.06 ± 0.01[b]	1.39 ± 0.02[a]
b^*	16.96 ± 0.04[a]	7.71 ± 0.06[b]
Chroma	17.25 ± 0.04[a]	7.77 ± 0.06[b]
Hue angle	92.49 ± 0.01[a]	83.59 ± 0.07[b]

Values are means and SD of triplicate determinations.
[ab]Means with different letters in each row are significantly different ($P < 0.05$).
ECSSH = emulsion containing catfish skin soluble hydrolysate; ECSISH = emulsion containing catfish skin insoluble hydrolysate.

Table 6 — Rheological properties of ECSSH and ECSISH.

	Viscosity	*n*	*K* (Pa.s^n)	σ_o (Pa)
ECSSH	0.06 ± 0.00[b]	0.64 ± 0.01[a]	0.43 ± 0.05[b]	0.89 ± 0.03[b]
ECSISH	0.12 ± 0.00[a]	0.41 ± 0.01[b]	2.45 ± 0.10[a]	1.39 ± 0.01[a]

Values are means and SD of triplicate determinations.
[ab]Means with different letters in each column are significantly different ($P < 0.05$).
ECSSH = emulsion containing catfish skin soluble hydrolysate; ECSISH = emulsion containing catfish skin insoluble hydrolysate.
n = flow index; K = consistency index; σ_o = yield stress.

Flow properties of emulsions containing CSSH and CSISH

The viscosity of the emulsion containing CSISH (ECSISH) was significantly higher than that containing CSSH (ECSSH) (Table 6). The flow index values (n) for ECSSH and ECSISH were both less than 1, which indicated that they were pseudoplastic fluids (Paredes and others 1989). Values for n ranging from 0.13 to 0.91 were reported for commercial mayonnaises and model mayonnaise systems (Dickie and Kokini 1983; Steffe 1992).

The consistency index (K) value of ECSISH was higher (2.45) than ECSSH (0.43). Higher K value of emulsion indicates a more viscous consistency (Paredes and others 1989). Emulsion made with CSISH protein powder had higher yield stress values ($P < 0.05$) than that made with CSSH (Table 6), which indicated ECSISH had greater resistance to flow. Yield stress values obtained in this study for emulsions made with ECSSH and ECSISH were lower than values reported for commercial emulsions by Steffe (1992). The wide ranges of n, K, and σ_o values were reported for emulsions by Dickie and Kokini (1983), Steffe (1992), and Sathivel and others (2005b), which could be due to different ingredients used to prepare the emulsion or different shear rate ranges used for rheological study (Sathivel and others 2005b).

Dynamic rheological tests could be used to characterize viscoelastic properties of emulsion. The frequency sweep test of the ECSSH and ECSISH is shown in Figure 1. The G' (an elastic or storage modulus) and G'' (a viscous or loss modulus) of the emulsion samples containing CSSH and CSISH were determined as a function of frequency (ω) at a fixed temperature of 25 °C. G' is a measure of energy recovered per cycle of sinusoidal shear deformation and G'' is an estimate of energy dissipated as heat per cycle (Rao 1999). ECSSH and ECSISH (Figure 1) showed a gradual increase in both the storage modulus and loss modulus with increasing frequency and both emulsions had higher G' than G''. The G' and G'' values for ECSISH were greater than those of ECSSH, which indicated that the viscoelastic characteristic of the ECSISH was greater than the ECSSH.

The oscillating stress test can indicate the structured stability of the emulsion. The oscillation stress sweep curves (Figure 2)

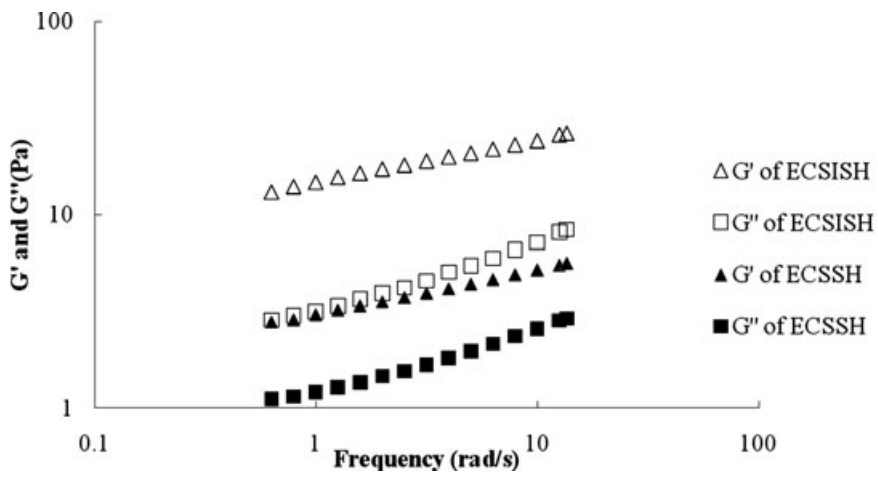

Figure 1 — Rheology properties of emulsions containing catfish skin hydrolysates. G' and G'' indicate storage and loss modulus, respectively. ECSSH = emulsion containing catfish skin soluble hydrolysate; ECSISH = emulsion containing catfish skin insoluble hydrolysate.

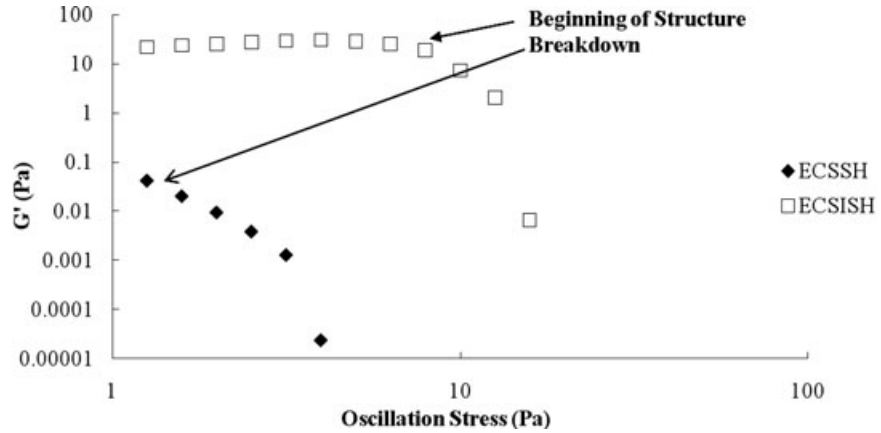

Figure 2—Rheological properties of ECSSH and ECSISH: oscillation stress sweeps. ECSSH = emulsion containing catfish skin soluble hydrolysate; ECSISH = emulsion containing catfish skin insoluble hydrolysate.

indicated where the structural breakdown of the emulsion occurred. Emulsion structure of the ECSISH broke down gradually with increased oscillation stress. The structure of ECSISH began to break down at about 8 Pa, while the structure of ECSSH started to break down at the beginning of the oscillation stress sweep (1 Pa). If the material structure is broken down suddenly after the linear region, the material has poor stability (TA Instruments 2002). In this oscillation-stress sweep study, emulsion made with CSISH had good stability and viscoelastic properties when high stress was applied on their structure. The dynamic rheological measurements may be a useful tool for evaluating emulsion quality.

Conclusions

Catfish skin was hydrolyzed and the soluble and insoluble fractions were separated and freeze dried. CSSH had significantly higher yield, protein, ash, and moisture content but lower fat content than that of CSISH. CSSH and CSISH exhibited differences in amino acid profiles and mineral contents. CSSH had higher nitrogen solubility and fat adsorption capacity than CSSIH. The emulsions made with the 2 catfish skin hydrolysates had viscoelasticity properties with $G' > G''$, which means that they could be used as a potential emulsifier and when used to produce an emulsion system showed pseudoplastic characters ($n < 1$). The emulsion made from CSSH exhibited a higher flow behavior index (n) and lower viscosity and consistency index (K), which indicates the ECSSH is less stable than the ECSISH. CSISH and CSSH have desirable functional and rheological properties. Protein hydrolysates made from catfish skin can be converted into a high value protein powder food ingredient. Application of these ingredients could include incorporation into muscle tissue products by injection, tumbling, and coating.

Acknowledgment

This study was financially supported by the Louisiana State Univ. Agricultural Center, USDA/CSREES Aquaculture Special Grant.

References

Althouse PJ, Dinakar P, Kilara A. 1995. Screening of proteolytic enzymes to enhance foaming of whey protein isolates. J Food Sci 60:1110–2.
[AOAC] Assoc. of Official Analytical Chemists. 1995. Official methods of analysis. 16th ed. Arlington, Va.: AOAC.
Bordenave S, Fruitier I, Ballandier I, Sannier F, Gildberg A, Batista I, Piot JM. 2002. HPLC preparation of fish waste hydrolysate fractions: effect on guinea pig ileum and ace activity. Biochem Biotechnol 32:65–77.
Chen KM, Decker EM. 1994. Endogenous skeletal muscle antioxidants. Crit Rev Food Sci Nutr 34:403–26.
Chobert JM, Bertrand-Harb C, Nicolas MG. 1988. Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. J Agric Food Chem 36:883–92.
Davis SS. 1973. Rheological properties of semisolid foodstuff. J Text Stud 4:15–40.
Dickie AM, Kokini JL. 1983. An improvement method for food thickness from non-Newtonian fluid mechanics in the mouth. J Food Sci 48:57–61, 65.
Doucet D, Gauthier FS, Otter ED, Foegeding AE. 2003. Enzyme-induced gelation of extensively hydrolyzed whey proteins by Alcalase: comparison with the plastein reaction and characterization of interactions. J Agric Food Chem 51:6036–42.
[FAO/WHO]. Food and Agriculture Org. /World Health Org. 1990. Protein quality evaluation. Report of a joint FAO/WHO expert consultation held in Bethesda, Md., 4–8 Dec.1989. FAO/WHO: Rome, Italy.
Feng J, Xiong YL. 2003. Interaction and functionality of mixed myofibrillar and enzyme-hydrolyzed soy proteins. J Food Sci 68:803–9.
Gauthier SF, Paquin P, Pouliot Y, Turgeon S. 1993. Surface activity and related functional properties of peptides obtained from whey proteins. J Dairy Sci 76:321–8.
Hoyle NT, Merritt JH. 1994. Quality of fish protein hydrolysates from herring (*Clupea harengus*). J Food Sci 59:76–9, 129.
Jost R, Monti JC, Pahud JJ. 1977. Partial enzymatic hydrolysis of whey protein by trypsin. J Dairy Sci 60:1387–93.
Kuipers BJH, Van Koningsveld GA, Alting AC, Driehuis F, Gruppen H, Voragen AGJ. 2005. Enzymatic hydrolysis as a means of expanding the cold gelation conditions of soy proteins. J Agric Food Chem 4:1031–8.
Lee SW, Shimizu M, Kaminogawa S, Yamaguchi K. 1987. Emulsifying properties of a mixture of peptides derived from the enzymatic hydrolysates of β-casein. Agric Biol Chem 51:161–5.
Lin S, Chiang W, Cordle CT, Thomas RL. 1997. Functional and immunological properties of casein hydrolysates produced from a two-stage membrane system. J Food Sci 62:480–3.
Mahmoud MI, Malone WT, Cordle CT. 1992. Enzymatic hydrolysis of casein: effect of degree of hydrolysis on antigenicity and physical properties. J Food Sci 57:1223–9.
Mendis E, Rajapakse N, Kim SK. 2005. Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J Agric Food Chem 53:581–7.
Min S, Mistry BS, Lee OH. 2003. Improvement of oxidative and emulsion stability of model salad dressing by glucose oxidase-catalase. J Food Sci 68:1272–5.
Morr V, German B, Kinsella JE, Regenstein JM, Van Buren JP, Kilara A, Lewis BA, Mangino ME. 1985. A collaborative study to develop a standardized food protein solubility procedure. J Food Sci 50:1715–8.
Nankervis L, Southgate P. 2009. Enzyme and acid treatment of fish meal for incorporation into formulated microbound diets for barramundi (*Lates calcarifer*) larvae. Aquac Nutr 15:135–43.
[NASS] National Agricultural Statistics Service. 2006. Catfish processing. Washington, D.C.: Agricultural Statistics Board, U.S. Dept. of Agriculture. Released on August 21, 2006.
Novo Nordisk Technical Bulletin. 1995. Alcalase® Food Grade. Bagsvaerd, Denmark: Enzyme Process Div., Novo Nordisk Co.
O'Donnell CD, Dornblaser L. 2002. Amino acids/peptides. Prep Food 117:72–3.
Ovissipour M, Abedian A, Motamedzadegan A. Rasco B, Safari R., Shahiri H. 2009. The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from Persian sturgeon (*Acipenser persicus*) viscera. Food Chem 115:238–42.
Paredes MDC, Rao MA, Bourne M. 1989. Rheological characterization of salad dressing. 2. Effect of storage. J Text Stud 20:235–50.
Petersen BR. 1981. The impact of the enzymatic hydrolysis process on recovery and use of proteins. In: Enzymes and food processing. London: Elsevier Applied Science Publishers. p 149–75.
Rao MA. 1999. Rheological of fluids and semisolids. In: Principal and applications. Gaitherburg, Md.: Aspen Publishers Inc.
SAS. 2002. SAS/STAT user's guide, version 8.2; Cary, N.C.: SAS Inst.
Sathivel S, Bechtel PJ, Babbitt J, Smiley S, Crapo C, Repond KD, Prinyawiwatkul W. 2003. Biochemical and functional properties of herring (*Clupea harengus*) byproduct hydrolysates. J Food Sci 68:2196–200.
Sathivel S, Bechtel PJ, Babbitt J, Prinyawiwatkul W, Negulescu II, Reppond KD. 2004. Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) byproduct. J Agric Food Chem 52:5040–6.
Sathivel S, Smiley S, Prinyawiwatkul W, Bechtel PJ. 2005a. Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. J Food Sci 70:C401–6.
Sathivel S, Bechtel PJ, Babbitt KJ, Prinyawiwatkul W, Patterson M. 2005b. Functional, nutritional, and rheological properties of protein powders from arrowtooth flounder and their application in mayonnaise. J Food Sci 70:E1–7.

Sathivel S, Huang J, Bechtel PJ. 2008. Properties of pollock (*Theragra chalcogramma*) skin hydrolysates and effects on lipid oxidation of skinless pink salmon (*Oncorhynchus gorbuscha*) fillets during 4 months of frozen storage. J Food Biochem 32:247–63.
Schagger H, Von Jagow G. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of protein in the range of 1 to 100 kDa. Anal Biochem 166:368–79.
Shahidi F. 1994. Seafood processing byproducts. In: Shahidi F, Botta JR, editors. Seafoods: chemistry, processing technology, and quality. London: Blackie Academic & Professional. p 321–34.
Shahidi F, Han XQ, Synowiecki J. 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem 53:285–93.
Shahidi F, Amarowicz R. 1996. Antioxidant activity of protein hydrolysates from aquatic species. J Am Oil Chem Soc 73:1197–9.
Sherman P. 1970. Industrial Rheology. London: Academic Press.
Steffe JF. 1992. Yield stress: phenomena and measurement. In: Singh RP, Wirakaratakusumah MA, editors. Advances in food engineering. London: CRC Press.
Sugiyama K, Takada K, Egawa M, Yamamoto I, Onzuka H, Oba K. 1991. Hypotensive effect of fish-protein hydrolysate. J Japan Soc Biosci Biotechnol Agrochem 65:35–43.
TA Instruments. 2002. Rheology technical library. New Castle, Del.: TA Instruments.
Theodore AE, Raghavan S, Kristinsson H. 2008. Antioxidative activity of protein hydrolysates prepared from alkaline-aided channel catfish protein isolates. J Agric Food Chem 56:7459–66.
Wardlaw GM, Insel PM. 1995. Perspectives in nutrition. 3rd ed. London: Mosby.
Wasswa J, Tang J, Gu X. 2008. Functional properties of grass carp (*ctenopharyngodon idella*), nile perch (*lates niloticus*) and nile tilapia (*oreochromis niloticus*) skin hydrolysates. Int J Food Prop 11:339–50.
Yang H, Wang Y, Jiang M, Oh JH, Herring J, Zhou P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*ictalurus punctatus*) skin gelatin. J Food Sci 72:C188–95.
Yatsumatsu K, Sawada K, Moritaka S. 1972. Whipping and emulsifying properties of soybean products. Agric Biol Chem 36:719–27.